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# Effects of prednisone withdrawal on the new metabolic triad in cyclosporine-treated kidney transplant patients

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*Background.* Cardiovascular disease is a major cause of morbidity and mortality after renal transplantation. Prednisone (Pred) maintenance therapy is associated with risk factors for atherosclerosis. Therefore, we were interested in quantifying the effects of Pred withdrawal on body weight and waist circumference as well as on metabolic markers of coronary heart disease risk.

*Methods.* Twenty-six cyclosporine-treated renal transplant patients (13 men and 13 women) were evaluated before and after at least 11 months (16 ± 2.9 months) of Pred withdrawal. A complete fasting lipoprotein-lipid profile as well as anthropometric measurements were obtained from each patient.

*Results.* Pred withdrawal was associated with a 6.0% reduction of body weight (−4.34 ± 5.40 kg; $P < 0.05$) and with a 7.7% decrease in waist girth (−7.13 ± 5.75 cm; $P < 0.005$) in women, whereas no change in these variables were observed in men. In both genders, plasma low-density lipoprotein (LDL) cholesterol and triglyceride concentrations were unaffected by Pred withdrawal, whereas plasma high-density lipoprotein (HDL) cholesterol levels decreased by 14.0% in women (−0.22 ± 0.22 mmol/L; $P < 0.005$) and 22.0% in men (−0.36 ± 0.28 mmol/L; $P < 0.005$). Pred withdrawal was associated with a significant reduction in plasma apolipoprotein B concentrations in both women (−0.28 ± 0.15 g/L; −24.6%; $P < 0.0001$) and men (−0.22 ± 0.19 g/L; −20.5%; $P < 0.005$). A significant reduction in fasting insulin was observed in both women (−27.8 ± 27.9 pmol/L; −25.3%; $P < 0.005$) and men (−25.0 ± 32.8 pmol/L; −21.4%; $P < 0.05$), whereas the LDL peak particle size was unaffected by Pred withdrawal.

*Conclusions.* Pred withdrawal modifies several anthropometric and metabolic cardiovascular risk factors in renal transplant patients. Furthermore, female patients may derive further benefits of Pred withdrawal resulting from the concomitant loss of body weight and abdominal fat.

**Key words:** immunosuppression withdrawal, body weight post-transplant, lipoprotein-lipid profile, atherogenic metabolic triad, hyperlipidemia, cardiovascular disease.

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Cardiovascular disease is the most common cause of death after renal transplantation [1–4]. One of the major metabolic risk factors for the development of cardiovascular disease is hyperlipidemia, which is a well-recognized complication in renal transplant recipients (RTX) [5–10]. Although several factors such as weight gain resulting from an increase in caloric intake, steroid-induced insulin resistance, hyperinsulinemia and diabetes, kidney graft dysfunction and concomitant antihypertensive medications have been documented to contribute to the expression of hyperlipidemia in RTX, there is evidence for a major contribution of the immunosuppressive therapy required in these patients [11–14].

After transplantation, elevated levels of serum total cholesterol, low-density lipoprotein (LDL) cholesterol, apolipoprotein (apo) B and triglycerides (TG) have been related to the combined use of cyclosporine (CsA) and prednisone (Pred) [7, 8, 15–20]. It is generally believed that CsA has adverse effects on the lipoprotein-lipid profile mainly through increases in total cholesterol, LDL cholesterol and apo B levels. Addition of Pred appears to induce a further increase of total cholesterol, which mainly results from the elevation of high-density lipoprotein (HDL) cholesterol and TG concentrations [15–17, 20, 21]. However, the respective contributions of these drugs to the altered metabolic risk factor profile remains uncertain and the withdrawal of steroids in CsA-treated patients has generated equivocal results. The reported benefits of steroid withdrawal in RTX included improved hypertension [22, 23], reduced cholesterol or LDL cholesterol and apo B levels [24–26], and enhanced glycemic control in diabetic patients [27, 28]. Moreover, after withdrawal, fewer antihypertensive drugs were necessary, fewer patients needed cholesterol-lowering drugs, and the frequency of type 2 diabetes was reduced [28]. One study has suggested beneficial effects of Pred on blood pressure, cholesterol levels and glycemia as well as on the occurrence of these metabolic alterations [28]. In the other hand, another study has reported potentially dele-

terious effects of tapering corticosteroids as suggested by a large fall in HDL cholesterol levels and increases in TG concentrations [29].

Furthermore, weight gain also has been reported in renal transplant patients under corticosteroid therapy [14]. Obesity, especially visceral obesity, is associated with an atherogenic fasting lipoprotein phenotype, which includes hypertriglyceridemia, elevated apo B levels, reduced HDL cholesterol concentrations and an increased proportion of small, dense LDL particles [30–32]. This atherogenic fasting lipoprotein phenotype of visceral obesity has been reported to be a feature of the insulin resistance syndrome, which is associated also with impaired insulin action and an elevated blood pressure [33, 34]. Increasing evidence suggests that the measurement and interpretation of some elements of this syndrome, which we have described as the atherogenic metabolic triad of unconventional risk factors (hyperinsulinemia, elevated apo B and small, dense LDL), may further improve the discrimination of individuals at high risk for cardiovascular disease [35].

Since the treatment of RTX with immunosuppressive drugs, especially corticosteroids, affects body weight, insulinemia and the plasma lipoprotein-lipid profile, it was relevant to verify whether the withdrawal of corticosteroids may affect components of the insulin resistance syndrome and more specifically, the new atherogenic metabolic triad: elevated insulin and apo B, and small dense LDL phenotype. Therefore, the objective of the present study was to quantify the effects of Pred withdrawal on morphometric and metabolic risk factors for cardiovascular disease in a sample of 26 RTX.

## METHODS

### Patients

Twenty-six RTX (13 men and 13 women), treated with CsA and Pred for immunosuppression, were followed in the present study. Subjects were first evaluated three to four years after renal transplantation and re-evaluated for cardiovascular risk factors following Pred withdrawal of about 16 months. All the kidney allografts came from cadavers. Neither acute allograft rejection nor significant leukopenia was observed following Pred withdrawal among these kidney transplant patients. However, almost half of the patients presented arthralgic pains that decreased with time. There was no wash-in period before the initiation of this study, but the results indicated that there was no significant change in the investigated parameters for the whole year prior to the start of the study. This study was the non-randomized arm of the Collaborative Transplant Study (CTS) [36]. To be included in the study, all patients had to have an excellent and stable renal function (creatinine $<180$ μmol/L), no significant proteinuria ($<300$ mg/day) and the panel of reactive antibodies had to be below 80%. Furthermore, to be eligible for the study, the transplantation procedure had to be performed at least six months prior to the tapering protocol of Pred and patients had to show no signs of allograft rejection. After meeting all of these inclusion criteria, patients were invited to participate to the study on a voluntary basis. The Pred was withdrawn according to the CTS protocol. Patients were on a double Pred and CsA therapy. At the time of the drug tapering, most of patients were on 10 mg or less of Pred. This drug was withdrawn progressively until complete withdrawal over a six-month period. Serum creatinine and CsA levels were measured every two weeks and 24-hour urinary samples were collected every month during the withdrawal period. Serum CsA levels remained stable (150 to 250 μg/L) throughout the tapering period and for the three following months. After the six-month withdrawal period, serum creatinine and CsA levels continued to be evaluated every two weeks for a period of two months, whereas the 24-hour urinary samples were collected every month for an additional four-month period. Patients with steroid-induced diabetes and with type 1 diabetes also were eligible to participate in the study in order to verify the potential effect of steroid withdrawal on their glucose control. Older patients were favored because they are at lower immunologic risk than young patients and because nephrologists felt that these older patients were at higher risk of side effects of steroids. After meeting all these criteria inclusion, patients were invited to participate to the study on a voluntary basis. One man and two women were current smokers. Ex-smokers who had stopped smoking for more than one year were considered to be non-smokers. Three women and two men were diagnosed as type 2 diabetic patients. Subjects with elevated cholesterol levels received HMG CoA-reductase inhibitors (8 men and 5 women) for whom doses remained stable for the entire study period. Regarding the intake of HMG CoA-reductase inhibitors the year before the instigation of the study, three patients had started hypolipidemic drugs, one had increased the dose whereas another had decreased the dose. Finally, one patient was switched from one statin to another. Patients with hypertension were usually treated with angiotensin-converting enzyme (ACE) inhibitors (10 men and 3 women) and/or diuretics (4 men and 7 women). This study was approved by the Medical Ethics Committee of the L'Hôtel-Dieu de Québec. Informed written consent was obtained from all participants.

### Measurement of body fatness and blood pressure

Body weight, height and waist circumferences were measured by following the procedures recommended at the Airlie conference [37]. More specifically, the measurement of waist circumference was performed at the narrowest part of the torso located between the lower

rib and the iliac crest while the subjects were standing, after a moderate expiration. Blood pressure was measured with a standardized sphygmomanometer after at least five minutes with the patients in a supine position.

### Laboratory methods

Venous blood samples were obtained after a 12-hour overnight fast. Cholesterol and TG levels were measured by enzymatic methods (International Laboratory Co., Lexington, MA, USA), creatinine (serum and urine) was determined by a colorimetric assay using alcalin picrate (Chiron, Cergy Pontoise, France) and urinary proteins were measured using the pyrogallol red assay (EMS Inc., Exton, PA, USA). All those assays were performed using a ILAB 900 analyzer (Beckman Coulter, Chaska, MN, USA). HDL cholesterol was determined by precipitation of apo B-containing lipoproteins using magnesium phosphotungstate (Fisher Scientific, Nepean, ON, CAN). LDL cholesterol was calculated according to the Friedewald formula if TG concentrations were below 4.5 mmol/L [38]. Apo B was determined by turbidimetry using human polyclonal antibodies (Atlantic Antibodies, Stillwater, MN, USA). Fasting insulinemia was determined in duplicates in frozen sera samples using a human insulin specific radioimmunoassay (RIA) kit (Linco Research Inc., St. Louis, MO, USA), showing little cross-reactivity (<0.2%) with human pro-insulin, as previously described [39]. Coefficients of variation for cholesterol, TG, HDL, apo B and fasting insulin levels were <2.5%, <4%, <6.5%, <4.5% and <7.5%, respectively.

### Assessment of cholesteryl ester transfer protein activity

Cholesteryl ester transfer protein (CETP) activity was measured by an isotopic transfer assay using diluted plasma with exogenous substrates [40, 41]. Recent studies have suggested that the assessment of CETP activity approximates CETP mass [40, 41].

Plasma CETP activity was measured as the relative transfer of total tritiated cholesteryl ester ($^3$H-CE) from $HDL_3$ (donor lipoprotein) to an acceptor lipoprotein fraction (d = 1.019 to 1.063 g/mL). Donor and acceptor lipoproteins were prepared from fresh plasma of normolipidemic volunteers. The CETP assay consisted of 50 μL of radiolabeled $HDL_3$, 200 μL of the acceptor fraction and 10 μL of plasma in a final volume of 270 μL, incubated for 16 hours at 37°C. Tris buffer was used as blank. Internal standards were included in each assay. The reaction was stopped by transferring the tubes to ice for 15 minutes. Donor and acceptor lipoproteins were separated by precipitation of apo B-containing lipoproteins with heparin (6%)/$MnCl_2$ (2.1 mol/L). Aliquots of the supernatant (200 μL) were transferred to plastic counting vials with liquid scintillant (Ecolite, ICN, Costa Mesa, CA, USA). The samples were counted in Wallac 1049 Liquid Scintillation Counter (Pharmacia, Turku, Finland) for two minutes with a counting error of less than 1%. CETP activity was expressed as the percentage of radioactivity transferred from $HDL_3$ to the acceptor fraction over 16 hours of incubation. All assays were performed as duplicates and were re-run when the coefficient of variation within duplicates exceeded 4.5%.

### Assessment of LDL size by gradient gel electrophoresis

Non-denaturating 2 to 16% polyacrylamide gradient gel electrophoresis (PAGG) was performed on whole plasma kept at −80°C before use, according to the procedure described by Krauss and Burke [42] and McNamara et al [43]. Gels were cast in our laboratory using acrylamide and bis-acrylamide (30:0.8) obtained from Bio-Rad (Hercules, CA, USA). A volume of 7.5 μL of plasma samples was applied on lanes in a final concentration of 20% sucrose and 0.25% bromophenol blue. Electrophoresis was performed in a refrigerated cell (10 to 15°C) for a pre-run of 15 minutes at 125 V and for the entry of samples into stacking at 70 V, followed by migration at 200 V for 12 to 16 hours and finally at 400 V for 2 to 4 hours. Gels were stained for lipids overnight with Sudan black (Lipostain, Paragon electrophoresis system; Beckman, Montréal, Canada) in 55% ethanol. Gels were destained in a 45% ethanol solution, and the original gel size was restored in a 9% acetic acid, 20% methanol solution. A plasma pool was used as an internal standard. Gels were analyzed using an optical densitometric image analyzer (Bio-Image Visage 110) coupled to a SPARC Station 2 Sun computer (Millipore, Ville St-Laurent, Canada) and using GEL 1D software. LDL peak particle size was obtained with the migration of standards of known diameter, such as ferritin (122 Å), thyroglobulin (170 Å), 380 Å latex beads (Duke Scientific Corporation, Palo Alto, CA, USA), and plasma standards of known diameters. Inter- and intra-assay coefficients of variation were <2% and <1.5%, respectively.

### Statistical analyses

Student paired *t* tests were performed to test the significance of changes in variables measured over the average 16-month period (range 11 to 22 months) of steroid withdrawal. Pearson correlation coefficients were used to quantify the associations among variables (*1*) at baseline and at follow-up and (*2*) between changes observed for variables studied over the 16-month period. All statistical analyses were performed on the SAS package (SAS Institute, Cary, NC, USA).

## RESULTS

Baseline clinical characteristics of the patients are shown in Table 1. All patients were under CsA and Pred

**Table 1.** Baseline clinical characteristics of 13 female and 13 male renal transplant patients before prednisone withdrawal

| Variables | Women | Men |
|---|---|---|
| Age *years* | 57.6 ± 5.84 | 57.2 ± 11.1 |
| Time since transplantation *years* | 4.64 ± 2.91 | 3.72 ± 2.46 |
| Prednisone/cyclosporine | 13/13 | 13/13 |
| Prednisone *mg/day* | 7.11 ± 2.72 | 9.21 ± 2.67 |
| Cyclosporine *mg/day* | 178.5 ± 61.5 | 209.6 ± 70.4 |
| Cyclosporine *μg/L* | 216.8 ± 39.4 | 222.5 ± 35.1 |
| Diabetes | 3 | 2 |
| Menopause/HRT | 13/3 | N/A |
| Smokers | 2 | 1 |
| Blood pressure *mm Hg* | | |
| Systolic | 151.2 ± 12.9 | 138.7 ± 17.7 |
| Diastolic | 88.8 ± 8.20 | 84.2 ± 9.18 |
| Creatinine *μmol/L* | 97.7 ± 24.8 | 114.7 ± 17.7 |
| Creatinine clearance *mL/sec/1.73 m*2* | 1.20 ± 0.33 | 1.15 ± 0.52 |
| Proteinuria *g/day* | 0.11 ± 0.09 | 0.16 ± 0.17 |

HRT is hormone replacement therapy.

therapy at the beginning of the study. Three women and two men were diagnosed with type 2 diabetes and all women were postmenopausal at the time of the investigation. All patients were characterized by a good renal graft function (plasma creatinine <200 μmol/L and proteinuria <1.5 g/day).

Table 2 shows the physical characteristics of the patients before and after Pred withdrawal. A significant body weight loss (−4.34 ± 5.40 kg, −6.04%, $P < 0.05$) was noted in women following Pred withdrawal whereas no change was observed in men. Accordingly, waist circumference and BMI were also significantly reduced in women but not in men in response to tapering the steroids (−7.13 ± 5.75 cm, −7.73%, $P < 0.005$; −1.72 ± 2.10 kg/m$^2$, −6.04%, $P < 0.05$ for waist girth and BMI, respectively).

The effects of Pred withdrawal on the plasma lipoprotein-lipid profile are shown in Table 3. In women, the only significant change was for HDL cholesterol concentrations that were reduced by 14% (−0.22 ± 0.22, $P < 0.005$) after withdrawal of steroids. Decreased HDL cholesterol concentrations were found also in men (−0.36 ± 0.28, −22.0%, $P < 0.005$) and this reduction in HDL cholesterol was greater in men than in women. Thus, despite a significant reduction in total cholesterol levels in men (−0.56 ± 0.83, −9.74%, $P < 0.05$), the marked reduction in HDL cholesterol led to an increase in the cholesterol/HDL cholesterol ratio in men (+0.54 ± 0.74, +18.3%, $P < 0.05$). Despite the fall in HDL cholesterol concentrations, it is important to point out that levels after the withdrawal of steroids remained above 1.4 mmol/L in women and 1.1 mmol/L in men, values that correspond to the 50$^e$ percentile values of their respective age and gender [44]. Plasma CETP activity was significantly increased in both genders after Pred tapering ($P < 0.05$).

The effects of steroids withdrawal on the components of the new atherogenic metabolic triad are presented in Figure 1. Significant and substantial decreases in plasma insulin (−27.8 ± 27.9 pmol/L, −25.3%, $P < 0.005$; −25.0 ± 32.8 pmol/L, −21.4%; $P < 0.05$ for women and men, respectively) and apo B (−0.28 ± 0.15 g/L, −24.6%, $P < 0.0001$; −0.22 ± 0.19 g/L, −20.5%, $P < 0.005$) levels were observed in both genders. No significant change in LDL peak particle diameter was noted.

We then examined whether weight loss was associated with changes in insulin and apo B concentrations. Pearson's correlation coefficients were not significant between weight loss and changes in plasma insulin and apo B levels in both genders (data not shown).

As fasting TG concentration is the best predictor of LDL peak particle size, Figure 2 illustrates the relationships between these two variables as well as the correlation between their changes in the overall study sample. Significant associations were found between plasma TG and LDL size before ($r = -0.62$, $P < 0.0007$) and after ($r = -0.62$, $P < 0.0008$) Pred withdrawal. Furthermore, changes in LDL peak particle size were significantly correlated with changes in plasma TG levels ($r = -0.51$, $P < 0.008$). Thus, as no significant change in TG levels were noted following Pred withdrawal, these results suggest that the lack of increase in LDL size in response to steroid tapering was due to the absence of overall change in fasting TG concentration in response to the protocol.

## DISCUSSION

It is well known that the incidence of cardiovascular diseases is high after renal transplantation and the factors responsible for atherosclerosis in RTX are not well understood. Many factors known to increase the risk of cardiovascular disease are commonly found in RTX. Among those, hyperlipidemia is a common metabolic alteration after renal transplantation [6, 7, 9, 10].

The pathogenesis of hyperlipidemia in renal transplant patients is also poorly defined. In this regard, it has been reported that immunosuppressive therapy may be one of the possible causes of the atherogenic dyslipidemia noted after renal transplantation, since it has an adverse effect on lipid metabolism [7–9]. However, there is disagreement on the nature and magnitude of lipoprotein changes induced by these drugs. In the present study, it was therefore of particular interest to determine whether Pred withdrawal may improve the plasma lipoprotein-lipid profile and more specifically, the components of the new atherogenic metabolic triad (insulin and apo B levels and small, dense LDL) in renal transplant subjects in whom Pred was stopped for at least 11 months.

It is well documented that corticosteroid hormones create an insulin-resistant state leading to hyperinsulinemia [45, 46]. In the present study, fasting insulin levels

**Table 2.** Physical characteristics of 13 female and 13 male renal transplant patients before and after prednisone withdrawal

| Variables | Before | After | Absolute difference | Relative difference % | *P* value |
|---|---|---|---|---|---|
| Women | | | | | |
| Weight *kg* | 63.7 ± 14.5 | 59.3 ± 11.1 | −4.34 ± 5.40 | −6.04 | <0.05 |
| Waist girth[a] *cm* | 88.5 ± 15.6 | 81.3 ± 13.4 | −7.13 ± 5.75 | −7.73 | <0.005 |
| BMI $kg/m^2$ | 26.3 ± 5.78 | 24.6 ± 5.06 | −1.72 ± 2.10 | −6.04 | <0.05 |
| Men | | | | | |
| Weight *kg* | 73.1 ± 6.91 | 73.4 ± 8.79 | 0.38 ± 3.66 | 0.42 | NS |
| Waist girth[a] *cm* | 94.3 ± 9.02 | 94.0 ± 11.8 | 0.46 ± 6.99 | 0.48 | NS |
| BMI $kg/m^2$ | 25.9 ± 2.68 | 26.0 ± 3.42 | 0.14 ± 1.32 | 0.42 | NS |

[a] 12 subjects; BMI is body mass index

**Table 3.** Fasting plasma lipoprotein-lipid profile as well as plasma CETP activity of the 13 female and 13 male renal transplant patients before and after prednisone withdrawal

| Variables | Before | After | Absolute difference | Relative difference % | *P* value |
|---|---|---|---|---|---|
| Women | | | | | |
| Cholesterol *mmol/L* | 5.91 ± 0.89 | 5.50 ± 0.90 | −0.40 ± 0.73 | −6.94 | NS |
| LDL chol *mmol/L* | 3.31 ± 0.78 | 3.11 ± 0.73 | −0.20 ± 0.74 | −3.64 | NS |
| HDL chol *mmol/L* | 1.66 ± 0.54 | 1.44 ± 0.58 | −0.22 ± 0.22 | −14.0 | <0.005 |
| Chol/HDL *chol ratio* | 3.84 ± 1.13 | 4.19 ± 1.24 | 0.35 ± 0.82 | 10.8 | NS |
| Triglycerides *mmol/L* | 2.08 ± 0.94 | 2.13 ± 0.95 | 0.05 ± 0.70 | 11.2 | NS |
| CETP activity[a] | 19.9 ± 6.86 | 26.6 ± 7.89 | 6.72 ± 7.30 | 46.3 | <0.05 |
| Men | | | | | |
| Cholesterol *mmol/L* | 5.33 ± 0.78 | 4.77 ± 0.89 | −0.56 ± 0.83 | −9.74 | <0.05 |
| LDL chol *mmol/L* | 3.06 ± 0.88 | 2.87 ± 0.76 | −0.19 ± 0.67 | −2.63 | NS |
| HDL chol *mmol/L* | 1.52 ± 0.37 | 1.16 ± 0.24 | −0.36 ± 0.28 | −22.0 | <0.005 |
| Chol/HDL *chol ratio* | 3.70 ± 0.96 | 4.24 ± 0.95 | 0.54 ± 0.74 | 18.3 | <0.05 |
| Triglycerides *mmol/L* | 1.67 ± 0.64 | 1.67 ± 0.50 | −0.004 ± 0.57 | 8.33 | NS |
| CETP activity[a] | 17.5 ± 6.93 | 22.2 ± 6.84 | 4.63 ± 4.79 | 46.3 | <0.05 |

Data are means ± SD.
[a] $^3$H-CE transferred/16 hours

were significantly reduced after Pred tapering in both genders. Results of the present study provide further support to the notion that there is a deleterious effect of Pred on fasting insulin levels. There has been an increasing interest in insulin and insulin resistance as potential major risk factors for ischemic heart disease (IHD) and as central components of a plurimetabolic syndrome that may contribute to the atherosclerotic process [34]. Hyperinsulinemia has been related to IHD risk in at least five prospective studies in men [39, 47–50]. However, whether there is an independent association between plasma insulin levels and IHD after control for the concomitant variation of other metabolic markers remains controversial. Previous studies had suggested that the risk associated with hyperinsulinemia was largely mediated by the concomitant dyslipidemia found in hyperinsulinemic men [39, 47–52]. However, we have recently reported, among non-transplant patients, that the risk of IHD related to hyperinsulinemia was largely independent of the concomitant dyslipidemic state [39].

Reported alterations in the plasma lipoprotein-lipid profile in RTX include increases in both total cholesterol and TG levels and elevated LDL cholesterol and apo B concentrations [7–9, 15–20], metabolic changes that contribute to an increased IHD risk. In accordance with these previous findings, apo B concentrations in the present study were significantly reduced after Pred withdrawal in both genders. Reductions of apo B levels also have been reported in diabetic RTX following Pred withdrawal [25]. Moreover, only among men of the study were lower total cholesterol levels observed following Pred withdrawal. Results of the present study confirm previous observations suggesting that withdrawal of steroid therapy is accompanied by a decrease in total cholesterol concentrations [25, 26, 53, 54]. On the other hand, Hollander and colleagues reported no significant change in total cholesterol levels [28], a finding concordant with our results in women. No change was noted for plasma TG concentrations in both men and women, probably because they were already in the normal range at baseline. Conflicting results regarding the effects of steroid withdrawal on TG levels have been reported as either no change [28] or increases in TG concentrations following Pred tapering [29].

Plasma HDL cholesterol levels have been reported to be low, normal or even slightly elevated after renal

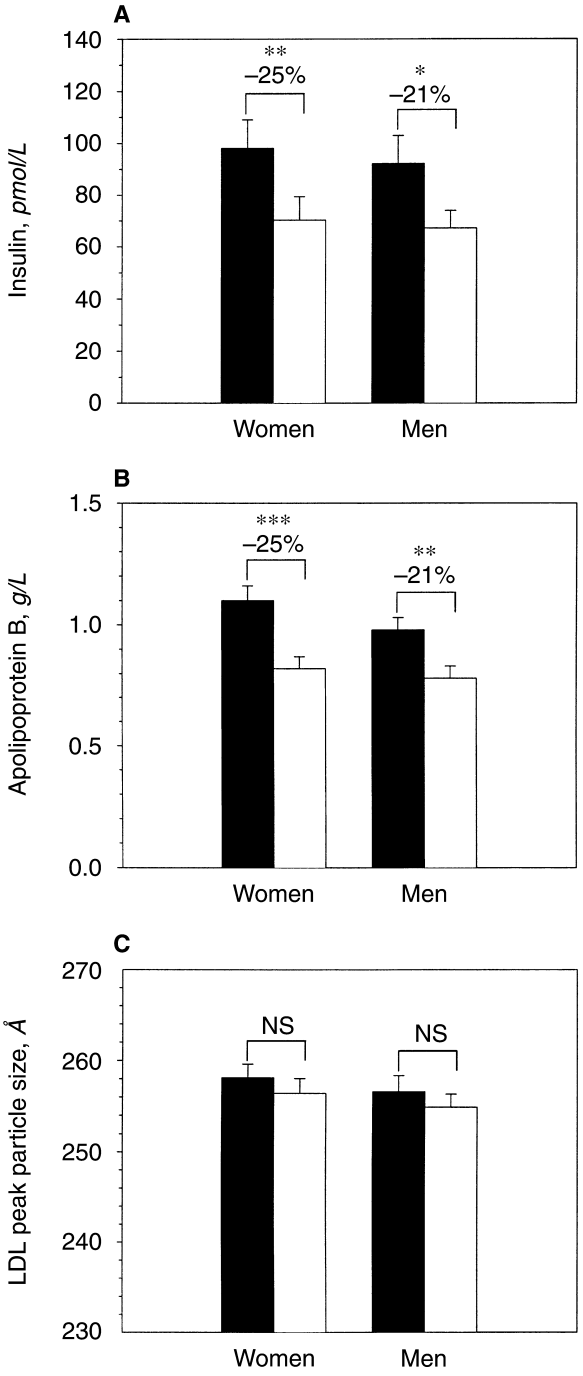


**Fig. 1. Fasting plasma insulin (*A*) and apolipoprotein B (*B*) levels as well as LDL peak particle size (*C*) in renal transplant patients before (■) and after (□) prednisone withdrawal.** The number above bars indicates the relative change when significant changes were noted. $^{*}P < 0.05$; $^{**}P < 0.005$; $^{***}P < 0.0001$; NS, non-significant.

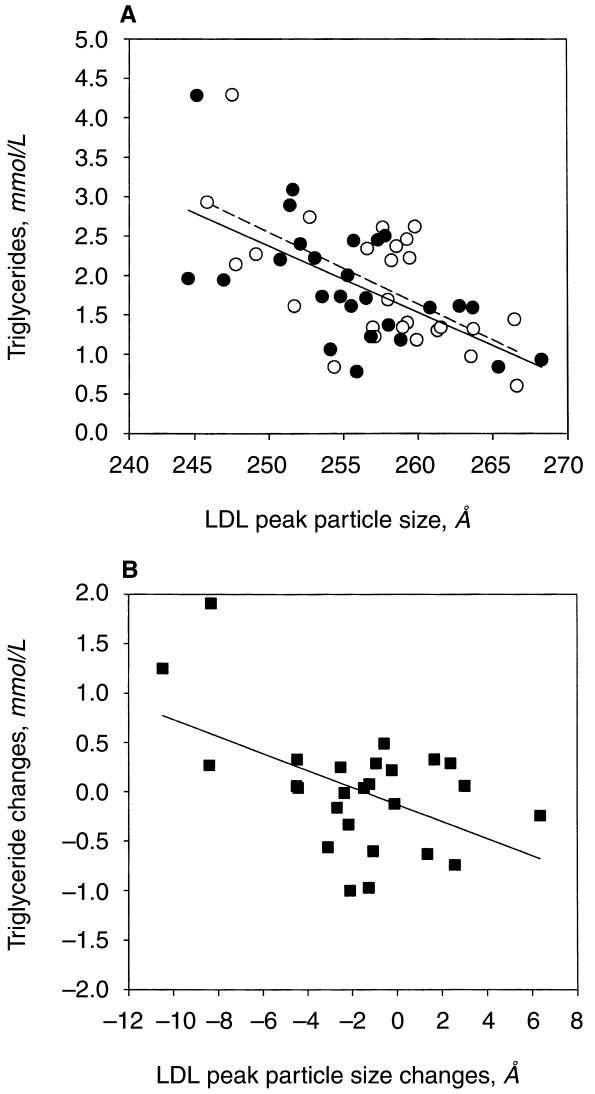


**Fig. 2. Associations between triglyceride levels and LDL peak particle size before (○) and after (●) prednisone withdrawal (*A*), and between triglyceride changes and LDL peak particle diameter changes (*B*) in response to prednisone withdrawal.** In panel A, before withdrawal, $r = -0.62$, $P < 0.0007$; after withdrawal, $r = -0.62$, $P < 0.0008$. In panel B, $r = -0.51$, $P < 0.008$;

transplantation [15–17, 20, 21]. Corticosteroids may raise HDL cholesterol levels by increasing apo A-I production by the liver or/and via a mechanism decreasing CETP activity. Indeed, corticosteroid therapy is associated with a reduction in plasma CETP in both normal subjects and in patients with the nephrotic syndrome [55], and with reduced liver CETP mRNA and plasma CETP in CETP transgenic mice [56]. In the present study, significantly reduced HDL cholesterol levels and higher plasma CETP activity were observed in both genders after Pred tapering. Similar results also were obtained by other groups who reported significant reductions in HDL cholesterol concentrations following Pred withdrawal [25, 28, 29]. The low CETP activity may explain the elevated HDL cholesterol levels observed after renal transplantation and the fall in HDL cholesterol concentrations following the withdrawal from steroids. HDLs are widely accepted to play a protective role against the development of

atherosclerosis [57, 58]. Whether these changes in HDL cholesterol levels associated with Pred withdrawal affect cardiovascular risk is not known, although it should be pointed out that values at the end of the follow-up period remained in a normal range. Moreover, although the decrease in HDL cholesterol produced an increase in the cholesterol/HDL cholesterol ratio in men, it should be emphasized that this ratio was quite low (below 4.5) and is still suggestive of a low cardiovascular disease risk [59]. Because a higher cholesterol/HDL cholesterol ratio is a risk factor for cardiovascular disease [60, 61], the net effect of the changes in the lipoprotein-lipid profile after Pred withdrawal on the risk of cardiovascular disease has led to conflicting interpretations and remains unresolved. We must keep in mind that no studies have documented an additional cardio-protective effect of increasing HDL levels with Pred therapy. Indeed, we frequently observed unusually high levels of HDL among these patients despite a well-established high prevalence of cardiovascular disease. Furthermore, members of CETP deficiency families carrying very high levels of HDL (in the range often found among Pred-treated patients) are not always protected, and they may even be at high risk for cardiovascular disease [62]. Therefore, it is still not clear whether elevated HDL levels carry the same cardio-protective effects in patients under Pred therapy as compared to those without it or after withdrawal.

There is evidence that the dense LDL phenotype is highly prevalent among IHD patients [63, 64]. This phenotype also is associated with concomitant variations in the plasma lipoprotein-lipid profile such as elevated TG levels and reduced HDL cholesterol concentrations [32, 63, 65], which are predictive of an increased IHD risk. However, in the present study, no improvement in the LDL peak particle size was observed with Pred withdrawal. This finding is not surprising because TG levels remained stable throughout the study. The significant negative association between changes in TG concentrations and changes in LDL peak particle size suggests that individuals who decreased their TG concentrations were those who showed an increase in LDL particle diameter.

A recent prospective study has emphasized the contribution of a new atherogenic metabolic triad (hyperinsulinemia, elevated apo B levels and small, dense LDL particles) as an important cluster of metabolic abnormalities predictive of a substantially increased risk of IHD [35]. Indeed, it has been reported that the risk of developing IHD over five years in a sample of initially asymptomatic middle-aged men was increased by more than 20-fold among individuals who were characterized by the atherogenic metabolic triad. Furthermore, the increased risk was not significantly modified by adjustment for classical lipid variables (LDL cholesterol, HDL cholesterol and TG levels) [35]. Results of this prospective study thus suggested that the measurement of fasting insulin and apo B concentrations and LDL particle diameter could provide further information on the risk of IHD compared to the estimated risk derived from the traditional lipid variables. The fact that we were able to favorably alter two of these three components (insulin and apo B levels) provides further support to the notion that we need to go beyond the assessment of classical metabolic risk factors such as lipoprotein-lipid levels to fully appreciate the benefits of Pred withdrawal on IHD risk.

A significant reduction in body weight has been reported following the withdrawal of corticosteroids [66], a finding concordant with our results in our female patients. Corticosteroids promote appetite, insulin resistance and hyperinsulinemia, alterations that may promote caloric consumption and weight gain in treated patients [46]. Previous studies of RTX have suggested that weight gain after transplantation is common, especially among women [67, 68]. In this regard, pharmacokinetic studies have shown that women presented relatively low clearance of methylprednisone, suggesting that higher exposure to steroids for any given dose could account for a greater weight gain observed in these women [69]. This biological variation among genders could, at least in part, explain differences found in our study between men and women regarding the effects of steroid withdrawal on body weight. For instance, the lowering of body weight after Pred withdrawal in women may have contributed to the greater reduction of fasting insulin and apo B levels and the smaller reduction of HDL cholesterol concentrations in women compared to men. It also is important to point out that as body fat distribution, especially visceral adipose tissue accumulation, has been shown to be more closely related to hyperinsulinemia and to an atherogenic dyslipidemia than excess fatness [70, 71], the possibility cannot be excluded that women of the present study had decreased levels of visceral adipose tissue following Pred tapering as estimated by reduced waist girth. This issue will have to be examined in future studies.

Despite new immunosuppressive protocols and a more effective management of risk factors, cardiovascular disease remains a major cause of morbidity and mortality after renal transplantation. A better understanding of the pathophysiology of the disease may lead to the development of effective strategies for prevention and management of coronary heart disease risk in RTX. Results of the present study suggest that Pred tapering may have beneficial effects on plasma lipoprotein levels as well as on components of the new atherogenic metabolic triad. Moreover, women may derive further benefits of steroid withdrawal resulting from the concomitant loss of body weight and abdominal fat. However, the benefits of Pred withdrawal have to be balanced against a higher risk of kidney rejection. In well selected individuals, our anthro-

pometric and metabolic data suggest that Pred withdrawal may have a beneficial impact on parameters that have been associated with an increased cardiovascular risk in the overall population, and such changes may contribute to decrease the cardiovascular disease risk in RTX.

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